REMARKS

Claims 1-13 are pending in the application. Claims 1, 2, 4, 6 and 10-13 have been amended. New claims 14-18. Support for new claim 14 can be found in claim 4 as originally filed. Support for new claims 15-17 can be found in claim 10 as originally filed. Support for new claim 18 can be found in the specification at page 6, line 2. Claims 2 and 12 have been amended to state that seeds produced by the method contain the heterologous gene and the selection marker. Claims 1, 2, and 12 have been amended to state that the transformed cells contain the heterologous gene and the selection marker. No new matter has been added.

At pages 2 and 3 of the Office Action, the Examiner objected to the Abstract, the drawings and the arrangement of the specification.

Applicants submit herewith a substitute Abstract.

With regard to the drawings, Applicants submit herewith substitute Figures 1 and 2 that are not in color for the Examiner's approval. Applicants also request an amendment to Figure 1A to correct a typographical error in plasmid pCH 73. HPPD pf W33 should be HPPD pf W336. The requested change is marked in red on the enclosed copy of Figure 1. Support for this amendment can be found in the specification at page 8, lines 15-32.

Section headings have been added in appropriate locations in the specification.

At page 3 of the Office Action the Examiner objected to claims 6-11 and 13 under 37 CFR 1.75(c) as being in improper multiple dependent format. Claims 4, 6, 10 and 13, which contained multiple dependencies as originally filed in French, were amended by Preliminary Amendment filed August 2, 2001 to delete the multiple dependencies. Claims 4, 6, 10 and 13 have been amended again to delete the multiple dependencies. Withdrawal of this objection to the claims is requested.

At page 4, the Examiner indicated that the specification contains essential material that is improperly incorporated by reference to a foreign patent application, patent or publication. The Examiner pointed to page 8, lines 5, 9, 10, 13, 18 and 20, and page 9, lines 19, 28, 29 and 30 of the specification.

Applicant's attorney, Liza D. Hohenschutz would like to thank Examiner David Fox for discussing this objection with her by telephone on January 31, 2003, and for withdrawing the objection. Applicants were notified that the objection was withdrawn by a facsimile copy of the Inverview Summary sent on January 31, 2003 by Examiner Fox.

At page 4 of the Office Action, the Examiner rejected claims 1-13 under 35 USC 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The rationale for this rejection is that Applicant is claiming genes for tolerance to HPPD inhibitors and because Applicant does not describe genes for tolerance to HPPD inhibitors other than the mutated DNA from *Pseudomonas fluorescens* in SEQ ID NO: 1 and SEQ ID NO: 2, it is not clear that Applicant was in possession of the invention as broadly claimed.

Applicants traverse this rejection. In order to satisfy the written description requirement of 112, first paragraph, the Applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the "written description" inquiry, whatever is claimed. *Vas Cath Inc. v. Mahurkur* 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). One shows that one is "in possession" of the invention by such descriptive means as words, structures, figures, diagrams, formulas, etc. that fully set forth the claimed invention. *Lockwood v. American Airlines* 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

Applicants respectfully submit that *University of California v. Eli Lilly*, cited by the Examiner, is not applicable to claims 1-13 and new claims 14-29. In *Amgen v. Hoechst Marion Roussel, Inc. and Transkaryotic Therapies, Inc.* (Case No. 01-1191, -1218) Fed. Cir. 2003, the Court indicated that *Eli Lilly* was inapposite to that case because the claim terms at issue, vertebrate or mammalian host cells, were not new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend." Similarly, Applicants are not claiming new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend. Applicants are not claiming genes for tolerance to HPPD inhibitors per se. Claims 1-13 and new claims 14-

18 are directed to methods of transforming plant cells and methods for preparing transgenic plants that employ a gene for tolerance to HPPD inhibitors.

Genes for tolerance to HPPD inhibitors are disclosed in the specification at page 7, lines 34-35, page 8, and page 9, lines 1-6 as comprising in the direction of transcription, a regulatory promoter sequence which is functional in plant cells and plants, functionally linked to a DNA sequence encoding an HPPD, functionally linked to a regulatory terminator sequence which is functional in plants. The HPPD sequence can be a native HPPD sequence from plants, microorganisms, fungi or mammals, or a mutated HPPD sequence. Examples of several HPPD sequences are provided by reference to publications in which the sequences are disclosed. The nucleotide sequence of the W336 mutant of *Pseudomonas fluorescens* HPPD is disclosed in SEQ ID NO: 1 and SEQ ID NO: 2. Regulatory promoters useful in plants are disclosed at page 11, lines 8-30. Terminator sequences functional in plants are disclosed at page 9, lines 3-7.

Applicants have conveyed with reasonable clarity to persons skilled in the art that they were in possession of the invention as of the filing date. Claims 1-13 and new claims 14-18 therefore comply with the written description requirement of section 112, first paragraph. Withdrawal of this section 112, first paragraph rejection is requested.

At page 5 of the Office Action, the Examiner rejected claims 1-13 under 35 USC 112, first paragraph as not enabled. The basis for the rejection is that, given the lack of guidance, the limited working examples in the specification, the breadth of the claims, and the unpredictability in the art, it would require undue experimentation for one skilled in the art to identify and isolate a multitude of non-exemplified genes for resistance to HPPD inhibitors and to transform and regenerate plants from non-exemplified monocotyledonous or dicotyledonous plants.

Applicants traverse this rejection. In order to make a rejection under section 112, first paragraph, the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement

requirement of section 112, first paragraph unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. It is incumbent upon the Examiner, whenever a rejection on this basis is made, to explain why he doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of his own with acceptable evidence or reasoning which is inconsistent with the contested statement.

In the present rejection, the Examiner relied upon U.S. Patent No. 4,443,971 (Chaleff; the '971 patent) and Chaleff et al, UCLA Symposium, Steamboat Springs, March 30-April 6, 1986 (Page 421, Mutation Breeding, lines 16-24) to show unpredictability in the art. These references, however, do not support the Examiner's assertion that the art relevant to the claimed methods is unpredictable. The disclosures of the references do not relate to the techniques employed in the claimed methods or the type of herbicide used in the claimed methods.

The '971 patent is directed to methods of producing plants having increased tolerance to a herbicide in which a plant cell or tissue culture is cultured in the presence of the herbicide for a time sufficient to kill a majority of the tissue or cell population, isolating tissue or cells tolerant of the herbicide and regenerating a plant from the tolerant tissue or cells. Prior to exposure of the plant cells or tissue to the herbicide, the tissue or cells can be subjected to mutagenesis to increase the possible number of herbicide-resistant cells. It is unclear how any unpredictability asserted by the Examiner in this method in any way relates to a method for transforming plant cells or a method of preparing transgenic plants.

Similarly, the Chaleff et al. publication does not support the Examiner's assertion that the art relevant to claims 1-13 is unpredictable. Chaleff et al. discloses strategies used to develop crop varieties resistant to sulfonylurea herbicides either by *in vitro* selection or by transformation with an altered form of the ALS gene. In the remarks in the present rejection relating to this publication, the Examiner stated that unpredictability is apparent since rare and resistant cells showing a resistant phenotype at the cellular level may not afford any regeneration into a whole plant because the resistant cells may

acquire other mutations at alleles that would interfere with the plants cells morphogenic properties and cited page 421, lines 16-24 of Chaleff *et al.*

Page 421, lines 16-24 of Chaleff *et al.* are in a section entitled Mutation Breeding, and Applicants submit that this assertion relates to mutation breeding, a technique not used the methods of the present invention. In mutation breeding, cells are subjected to mutagenesis using for example radiation or mutagenic chemicals prior to culture in the presence of the herbicide to increase the chances of finding herbicide resistant cells. Such cells may acquire mutations at any number of alleles because the mutagenic techniques randomly alter DNA. Moreover, claims 1-13 and new claims 14-29 are directed to methods that employ HPPD inhibitors, not sulfonylurea herbicides.

The copy of Chaleff *et al.* provided with the present Office Action only contains the odd numbered pages. The section on Mutation Breeding cited by the examiner runs at least to the next page, page 422 which was not available to Applicants. If the Examiner continues to rely upon this reference, the Examiner is requested to provide a copy of the entire publication to Applicants.

Claims 1, 4-11 and 14-18 are drawn to methods of transforming plant cells. Applicants described suitable genes for tolerance to HPPD inhibitors in the specification as well as suitable methods for transforming plant cells and examples of heterologous genes. The claimed method is exemplified in the specification in Example 2 with soy bean cells. Claims 2, 3, 12 and 13 are drawn to a methods for preparing transgenic plants wherein the transformed tissue used to regenerate the plants is produced using the claimed method of transforming plant cells. Example 2 in conjunction with Example 1 exemplify preparation of transgenic soy bean plants.

The Examiner's assertions that it would require undue experimentation to identify and isolate a multitude of non-exemplified genes for resistance to HPPD inhibitors, transform cells and regenerate plants from non-exemplified monocotyledonous and dicotyledonous plants are not supported by any reasoning or evidence. The Examiner's assertions of unpredictability in the art relate to a technique not used in the claimed methods. The Examiner has thus provided no reasonable basis for doubting the enablement of the claims. Claims 1-13 and new claims 14-18 must be taken as being in

compliance with section 112, first paragraph. Withdrawal of this section, 112, first paragraph rejection is requested.

At page 7 of the Office Action, the Examiner rejected claims 2-11 under 35 USC 112, second paragraph, as being indefinite. The phrase " characterized in that it consists in carrying out" was considered to be awkward and the Examiner suggested replacing it with -- and further comprising --. Claim 2 has been amended to delete the foregoing phrase and insert the phrase suggested by the Examiner.

The term "in particular" in claims 4 and 10 was objected to by the Examiner. Claims 4 and 10 have been amended to delete "in particular" and following material and present the deleted material in new claims. New claim 14 depends form claim 4 and is drawn to the types of plant cells deleted from original claim 4. New claims 15-17 depend from claim 10 and are drawn to the HPPD inhibitors deleted from original claim 10.

The term "more preferably" in claim 11, line 9 was objected to as indefinite as well as "[lacuna]" in line 10. The phrase "more preferably between 1 mg/ml and [lacuna] mg/ml" has been deleted from claim 11 and the deleted material presented in new claim 18. In new claim 18, the term "1 mg/ml" finds support in the specification at page 6, line 2.

At page 8 of the Office Action, the Examiner rejected claims 2 and 12 under 35 USC 101 because the claimed invention is directed to non-statutory subject matter. The basis for this rejection is that the first generation transgenic plants, because they are hemizygous for the transgene, would generate gametes not carrying the transgenic material. Thus, seeds of the claimed invention would comprise non-transformed material and thereby read upon a product of mature. The Examiner suggested that the claims be amended to recite -- seeds comprising the transgene--.

Applicants traverse this rejection. Step c) of claim 2 and step e) in claim 12 have been amended to state that the seeds comprise the heterologous gene and the selection marker. The seeds in claims 2 and 12 as amended do not read upon a product of nature. Withdrawal of this section 101 rejection is requested.

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Respectfully submitted,
CONNOLLY BOVE LODGE & HUTZ LLP

Date: Feb. 7, 2003

By: Liza D. Hohenschutz

Liza D. Hohenschutz
Reg. No. 33,712
P.O. Box 2207
Wilmington, Delaware 19899
Attorney for Applicants

Marked Up Version Of Amended Claims

1. (amended) Method for transforming plant cells by introducing a heterologous gene into said plant cells with a gene for tolerance to HPPD inhibitors as a selection marker, said method comprising the steps of:

a) preparing and culturing competent plant cells capable of receiving the heterologous gene and selection marker in a suitable medium,

b) transforming the competent cells with the heterologous gene and the selection marker,

c) growing and selecting the transformed cells comprising the heterologous gene and the selection marker in a suitable medium,

characterized in that a step for bleaching the competent plant cells is carried out before the transformation step (b), by introducing a suitable amount of HPPD inhibitor into the suitable culture medium of the competent plant cells.

2. (amended) Method for preparing transgenic plants comprising a heterologous gene integrated into their genome, comprising a method for transforming plant cells according to Claim 1, [characterized in that it consists in carrying out] and further comprising the following steps of :

d) regenerating plants from the transformed cells selected in one or more suitable media and, where appropriate,

e) producing and recovering [the] seeds of the fertile transformed plants, said seeds comprising the heterologous gene and the selection marker.

4. (amended) Method according to one of [Claims 1 to 3] Claim 1, characterized in that the plant cells are chosen from the cells of dicotyledonous plants[, in particular tobacco, rapeseed, sugar beet, potatoes, cotton and soya bean].

6. (amended) Method according to one of [Claims 1 to 5] Claim 1, characterized in that the competent plant cells are chosen from embryogenic calluses, cell cultures or a solid support or in suspension, or embryonic tissues.

10. (amended) Method according to [one of Claims 1 to 10] Claim 1, characterized in that the HPPD inhibitor is chosen from isoxazoles, [in particular isoxaflutole,] diketonitriles, [in particular 2-cyano-3-cyclopropyl-1-1(2-CH3SO2-4-CF3 phenyl)propan-1,3-dione and 2-cyano-3-cyclopropyl1-1-(2-CH3SO2-4-2-C12 phenyl)propan-1,3-dione,] triketones, [in particular sulcotrione or mesotrione,] and pyrazolinates.

11. (amended) Method according to Claim 10, characterized in that the concentration of HPPD inhibitors is between 0.5 mg/ml and 50 mg/ml[, more preferably between 1 mg/ml and [lacuna] mg/ml].

12. (amended) Method for preparing transgenic plants comprising a heterologous gene integrated into their genome, which method comprises a method for transforming plant cells by introducing a heterologous gene into said plant cells with a gene for tolerance to HPPD inhibitors as a selection marker, said method comprising the steps of:

a) preparing and culturing competent plant cells capable of receiving the heterologous gene and the selection marker in a suitable medium,

b) transforming the competent cells with the heterologous gene and the selection marker,

c) growing and selecting the transformed cells comprising the heterologous gene and the selection marker in suitable medium,

d) regenerating plants from the transformed cells selected in one or more suitable media and, where appropriate,

e) producing and recovering [the] seeds of the fertile transformed plants, said seeds comprising the heterologous gene and the selection marker, then producing novel

varieties of transgenic plants which have stably integrated the heterologous gene into their genome, in conventional selection programmes,

characterized in that a step for bleaching the competent cells is carried out before the transformation step (b), by introducing a suitable amount of HPPD inhibitor into the suitable culture medium of the competent plant cells.

13. Method according to [one of Claims 2 to 12] claim 2, characterized in that the selection marker gene is eliminated by crossing the transformed plants comprising the heterologous gene and the selection marker gene with a nontransformed variety of the same plant.

Marked Up Amended Pages of the Specification

Page 1, second paragraph (lines 11-26):

BACKGROUND OF THE INVENTION

Many techniques for transforming plant cells and plants by genetic engineering have been developed and thoroughly described in the literature. Firstly, methods which seek to introduce a DNA fragment carrying the heterologous gene in the form of naked DNA may be distinguished. This involves, in particular, bombarding cells, protoplasts or tissues with particles to which the DNA sequences are attached. Other methods may be used, such as microinjection or electroporation, or alternatively direct precipitation using PEG. Secondly, methods consisting in using, as a means of transfer into the plant, a heterologous chimeric gene in an agrobacterium tumefaciens Ti plasmid or an agrobacterium rhizogenes Ri plasmid will be distinguished. Those skilled in the art will choose the suitable method depending on the nature of the plant cell or of the plant to be transformed. Mention will in particular be made of the following Patents and Patent Applications: US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073, EP 267,159, EP 604 662, EP 672 752, US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442 174, EP 486 233, EP 486 234, EP 539 563, EP 674 725, WO 91/02071 and WO 95/06128.

Page 3, fourth paragraph (lines 23-30):

SUMMARY OF THE INVENTION

The present invention consists in improving such a use in such a way as to facilitate the process for identifying and selecting the transformed cells. A second object of the present invention consists in decreasing the time required for selecting the transformed plants and for producing fertile regenerated plants. Specifically, the general process for transforming, selecting, regenerating and recovering the seeds of fertile transformed plants may take several months depending on the plants under consideration, about 10 to 18

months in particular for plants such as soya bean. Decreasing this duration by one or more months constitutes a definite technological and economical advantage.

Page 4, fourth paragraph (lines 24-27):

DETAILED DESCRIPTION OF THE INVENTION

The plant cells according to the invention may be plant cells from monocotyledonous or dicotyledonous plants, more particularly crop plants which may or may not be intended for animal or human food, preferably dicotyledonous plants, in particular tobacco, rapeseed, sugarbeet, potatoes, cotton or soya bean, preferably soya bean.

Page 14, third full paragraph Page 14 (lines 7-13):

In order to avoid handling tissues and to gain time, the bombarded calluses are placed on sterile gauze screen fixed with two metal rings [(Figure 2)] which enable direct contact between the embryogenic tissues and the solid medium. The gauze screens are transferred onto fresh media every 15 days until green calluses are observed. It is understood that the principle of callus transfer described above is not limited to soya bean calluses and to selection with hygromycin, but may be used for any method for culturing tissues and cells suspensions which requires frequent changing of culture medium.

Page 15, third paragraph (lines 9-17):

10 to 15 days prior to the bombardment, the isoxaflutole or the diketonitriles are introduced into the D20 medium, at the abovementioned concentrations, so as to bleach the tissues. After bombardment, the tissues are placed directly in the same D20 medium comprising 2 mg/ml of isoxaflutole or diketonitrile (between 0.5 and 5 mg/l) and transferred into fresh medium every 15 days. After 4 transfers onto the isoxaflutole, green calluses are identified and amplified as described in Example 1. The time required for producing calluses of cells which are competent for the bombardment is 3 and a half months. The selection of the transformed cells (green calluses) occurs approximately 6 months after the initiation of the calluses for the transformation [(Figure 3)].

Page 16, seventh paragraph (lines 25-28):

The time required for producing calluses from cells which are competent for the bombardment is 2 months, the transformed green calluses being selected approximately 3 months after the initiation of the calluses for transformation [(Figure 3)].

Page 17, second paragraph (lines 7-10):

The use of the FNL medium in combination with the prior bleaching of the tissues according to the invention makes it possible to substantially decrease the time required for selecting the green calluses and the work load for the entire process of transforming the plants (Figure 2).

Marked Up Version Of The Abstract

[**Use of HPPF inhibitors as selection agents in the transformation of plants**

Abstract:

The present invention relates to a method for transforming plant cells by introducing a heterologous gene into said plant cells with a gene for tolerance to HPPD inhibitors as a selection marker, said method comprising the steps of:

a) preparing and culturing competent plant cells capable of receiving the heterologous gene n a suitable medium,

b) transforming the competent cells with the heterologous gene and the selection marker,

c) growing and selecting the transformed cells comprising the heterologous gene in a suitable medium,

characterized in that a step for bleaching the competent plant cells is carried out before the transformation step (b), by introducing a suitable amount of HPPD inhibitor into the suitable culture medium of the competent plant cells.]

ABSTRACT

The present invention provides a method for transforming plants cells by introducing a heterologous gene into competent plant cells with a gene for tolerance to HPPD inhibitors as a selection marker wherein a step for bleaching the competent plant cells is carried out prior to transforming the cells by introducing a suitable amount of HPPD inhibitor into the cell culture medium. The invention also provides methods for preparing transgenic plants comprising a heterologous gene.

ABSTRACT

The present invention provides a method for transforming plants cells by introducing a heterologous gene into competent plant cells with a gene for tolerance to HPPD inhibitors as a selection marker wherein a step for bleaching the competent plant cells is carried out prior to transforming the cells by introducing a suitable amount of HPPD inhibitor into the cell culture medium. The invention also provides methods for preparing transgenic plants comprising a heterologous gene.